Physical activity and cardiometabolic health

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CHAPTER 2

Physical activity, fatty liver and glucose metabolism over the life course: The Lifelines Cohort

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ABSTRACT

Objectives: We examined the dose-dependent association of habitual moderate-to-vigorous physical activity (MVPA) with the biochemical markers for non-alcoholic fatty liver disease (NAFLD) and whether this association changes with age and degree of impaired glucose metabolism. We also investigated whether the associations depend on the domain of MVPA.

Methods: In this study, using data from the population-based Lifelines Cohort (N=42,661), MVPA was self-reported on the short questionnaire to assess health-enhancing physical activity. NAFLD was defined as a fatty liver index value of (FLI)>60, based on body mass index, waist circumference, plasma triglycerides, and gamma-glutamyltransferase. Glucose metabolism was defined as normal (NGM), impaired (IGM), and type 2 diabetes mellitus (T2DM). Exclusion criteria were previously diagnosed hepatitis or cirrhosis and excessive alcohol use. All analyses were adjusted for age, sex, and education.

Results: Higher MVPA was dose-dependently associated with lower risk of having NAFLD: compared with "No-MVPA," the odds ratio (ORs) (95% confidence intervals) for MVPA quintiles were 0.78 (0.71;0.86), 0.64 (0.58;0.70), 0.53 (0.48;0.59), 0.51 (0.46;0.56), and 0.45 (0.41;0.50) for the highest level of MVPA. The association between MVPA and NAFLD was stronger for more impaired glucose status (OR_{NGM}=0.49 (0.42;0.57), OR_{IGM}=0.46 (0.40;0.54), OR_{T2DM}=0.42 (0.27;0.66))) and for older age ($OR_{20\text{-}40\ years}$=0.51 (0.42;0.62), $OR_{60\text{-}80\ years}$=0.37 (0.29;0.48)) with the highest level of MVPA, relative to No-MVPA. No favorable association was observed for occupational MVPA. With regard to MVPA and fibrosis, associations with fibrosis markers showed contradictory results.

Conclusion: Higher MVPA levels are dose-dependently associated with a lower NAFLD risk. This association is stronger in people with diabetes and older adults.

Keywords: Non-alcoholic fatty liver disease, physical activity, fatty liver index, diabetes, occupational physical activity

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is characterized by increased hepatic triglyceride accumulation in the absence of excessive alcohol consumption. This condition is a precursor of other liver pathological conditions, including steatohepatitis, fibrosis (FB), liver cirrhosis, and liver failure or hepatocellular carcinoma[1]. Furthermore, NAFLD has become more prevalent globally, affecting approximately 25% of the general population[2]. This has generated a need to investigate tools for improving the management of lifestyle or other factors.

Physical activity is regarded as a foundation for managing NAFLD [1, 3-4]. However, most reports on the benefits of physical activity with regard to NAFLD have been based on experimental studies[5-7]. Observational studies have identified lower levels of physical activity as a risk factor for developing NAFLD, suggesting that daily-life physical activity should be increased to prevent NAFLD [8-12]. Most studies consider only small sample sizes [8-11], and few have established any dose-dependent NAFLD risk reduction for increased physical activity [12-13]. Moreover, little is known about the potential benefits of moderate-to-vigorous physical activity (MVPA) within the context of total daily-life physical activity, which includes a variety of domains (e.g., occupational and non-occupational) that might play different roles in health [11]. It is therefore important to gather evidence to support the dose-dependency of the beneficial effects of physical activity and to determine whether such dose-dependency is related to specific domains.

The prevalence of NAFLD increases with age, due to age-related metabolic changes such as fat distribution from subcutaneous to ectopic sites, including liver and specific age-related hepatic changes [14-15]. In addition, type 2 diabetes mellitus (T2DM) is closely associated with the presence of NAFLD, with its incidence estimated to be around 70% in people with T2DM [16-18]. Studies have also indicated that older age and T2DM are associated with advanced progress of other pathological conditions, such as FB [19-20]. To date, no studies have investigated whether physical activity becomes more important role with age and impaired glucose metabolism, or whether it becomes less important. On the one hand, benefits may increase with age, but on the other hand, the effect of physical activity could be potentially outweighed by clinical factors (e.g., comorbidities and medication use).

The primary objective of this study was to examine the association of daily-life moderate-to-vigorous physical activity with the biomarkers of NAFLD – fatty liver index (FLI) and alanine aminotransferase (ALT); aspartate aminotransferase (AST); alkaline phosphatase (ALP); and gamma-glutamyltransferase (GGT) – in a large population-based cohort. A second objective was to evaluate how this association is altered in individuals with impaired glucose metabolism (IGM) and diabetes, as well

as across different age groups. The study also examined whether the associations depend on the domain of physical activity and how physical activity is related to the risk of FB in individuals with NAFLD.

METHODS

Data source and study population

Lifelines is a multidisciplinary prospective population-based cohort and biobank of more than 167,000 people living in the North of the Netherlands[21]. It uses a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical, and psychological factors that contribute to the health and disease of the general population, with a special focus on multi morbidity and complex genetics. The study was conducted according to the Helsinki Declaration, and it was approved by the medical ethical committee of the University Medical Center Groningen in the Netherlands. All participants provided their written informed consent [21-22].

In this cross-sectional study, the analyses were based on data available in June 2016 (n=57,774). From this population, we included subjects of Western European origin [23] between the ages of 18 and 80 years. The first exclusion criterion was any missing and/or implausible data related to the main outcomes: definition of the NAFLD and glucose status, and the assessment of physical activity. Further exclusions included excessive alcohol use (alcohol consumption>30g/day for males and 20g/day for females [1]), previously diagnosed hepatitis and/or cirrhosis, acute liver diseases (liver enzyme values>3 times the upper reference limit, i.e., for AST>120 U/L, ALT>135 U/L and GGT>165 U/L), Type 1 DM, current cancer, and diseases that impair or prevent participation in exercise (heart failure and renal failure). In all, 42,661 participants were included in the current analyses (**Figure S1**).

Anthropometry and laboratory tests

Body weight, height, waist circumference, and blood pressure were measured by a permanent staff of well-trained technicians using a standardized protocol [21]. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Fasting plasma glucose (FPG) was measured by the hexokinase method, and HbA1c was measured using high-performance liquid chromatography. Liver blood tests were measured routinely according to the recommendations of the International Federation of Clinical Chemistry on a Roche Modular platform. Measurements of ALT and AST were taken using pyridoxal phosphate activation. Total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol were

measured using an enzymatic colorimetric method, and triglycerides (TG) were measured using a colorimetric UV method, all on a Roche Modular P chemistry analyzer[21-22].

Assessment of physical activity

Physical activity was assessed using the short questionnaire to assess health-enhancing physical activity (SQUASH), which estimates habitual physical activities with reference to a normal week[24]. The SQUASH is pre-structured into four domains: commuting, leisure time and sports, household, and occupational activities. Questions consisted of three main queries: days per week, average time per day, and intensity. The SQUASH has been validated in the general population[24].

In this study, we used activities at the moderate (4.0-6.5 MET) to vigorous (≥ 6.5 MET) level. Metabolic equivalent (MET) values were assigned to activities according to Ainsworth's Compendium of Physical Activities[25]. Outcomes were presented as MVPA minutes per week (min/week). Participants were divided into six distinct categories based on the amount of total and non-occupational MVPA. Individuals who performed no physical activity at a moderate-to-vigorous level were considered inactive and classified as "No-MVPA." The other participants (MVPA>0 min/week) were divided into quintiles of MVPA, ranging from low (quintile 1, MVPA-Q1) to high (quintile 5, MVPA-Q5). The MVPA min/week (median, 25th and 75th percentile of MET/min/week) was used to define the total MVPA quintiles: 1-135 (420, 3.5-839), 136-269 (1200, 840-1679), 270-480 (2220, 1680-3000), 481-1105 (1640, 3001-5940), 1106-6840 (9000, 5942-31020). The following quintiles were defined for non-occupational MVPA: 1-90 (400, 3.5-585), 91-181 (840, 586-1080), 181-292 (1418, 1081-1810), 293-464 (2310, 1812-3023), 465-1150 (4367, 3024-28752), based on the min/week (median, 25th and 75th percentile of MET/min/week), respectively.

Assessment of NAFLD

The fatty liver index (FLI), a non-invasive marker for liver steatosis, was used to define NAFLD:

$$FLI = \left(\frac{e^{[0.953 \times \ln(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \ln(\text{GGT}) + 0.053 \times \text{WC} - 15.745]}}{1 + e^{[0.953 \times \ln(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \ln(\text{GGT}) + 0.053 \times \text{WC} - 15.745]}}\right) \times 100$$

where triglycerides are measured in mg/dl, GGT in IU/l, WC in cm and BMI in kg/m^2. Values of FLI>60 indicate the presence of NAFLD with an accuracy of 0.84, a sensitivity of 61%, and a specificity of 86%, as determined by ultrasonography[26].

Assessment of glucose metabolism

The following definitions were used in assessing glucose metabolism according to reports from the WHO/IDF consultation and the European Diabetes Epidemiology Group: normal glucose metabolism (NGM) – FPG<6.1 mmol/L or HA1C<5.7%, IGM – FPG between 6.1 to 6.9 mmol/L or HA1C between 5.7% and 6.4%, and diabetes – FPG≥7.0 mmol/L or HA1C≥6.5%, or self-reports of diagnosis by a physician, or the use of glucose-lowering agents [27-28].

Statistical analysis

The study characteristics were expressed as means with a standard deviation for normally distributed variables or as medians with interquartile range for non-normally distributed variables and numbers with percentages referring to the presence of NAFLD. The differences between groups were compared using Student *t* test or the Mann-Whitney *U* test for continuous variables. The frequency distributions of categorical variables were analyzed using the Pearson χ^2 test.

Binary logistic regression analysis was performed to evaluate the association between MVPA and NAFLD. Odds ratios (OR) are reported with 95% confidence intervals. Analyses were adjusted for age, sex, education (model1), daily caloric intake, and smoking (model2). The determinants consisted of six categories of MVPA, with No-MVPA as the reference group for regression analysis. Given that obesity may reflect general adiposity and, to a lesser extent, specific liver-fat deposition, linear regression was performed for the individual FLI components and other liver blood tests (ALT, AST and ALP). The variables in these linear regression analyses were first log-transformed to obtain normal distributions. The association between MVPA and fibrosis was investigated using the continuous scores of the NAFLD Fibrosis Score (NFS), FiB-4, and the AST-to-platelet ratio index (APRI) (Supplementary method) [29].

The study population was categorized according to glucose status (NGM, IGM and T2DM) and age (18-40, 40-60 and 60-80 years).

To study the risk of inactivity in sensitivity analysis, we used the first quintile of MVPA (MVPA-Q1) as a reference group. We also performed the regression analysis for the various levels of alcohol consumption, including the initially excluded excessive alcohol users. Finally, to compare the results of total daily-life MVPA, we analyzed time spent engaging in sports, which is more repetitive than other activities and therefore easier to report.

All statistical analyses were performed using IBM SPSS V.22.0 (Chicago, IL) and GraphPad Prism V.4.03 (San Diego, CA). A 2-sided statistical significance was set at $P < 0.05$ for all tests.

RESULTS

People with FLI≥60 (suspected NAFLD) accounted for 21.4% of the total population. Participants with NAFLD were older and more likely to be males with lower levels of education (**Table 1**).

Table 1. General characteristics of the study population

Characteristics	Total (n=42,661)	No NAFLD (n=33,580)	NAFLD (n=9,081)	*P* value*
Age (years)	44 (36-51)	43 (35-50)	47 (40-55)	**<0.001**
Male gender, *n* (%)	16,871 (39.5)	11,439 (34.1)	5,432 (59.8)	**<0.001**
Education: Low, *n* (%)	12,188 (29.2)	8,677 (26.4)	3,511 (39.7)	**<0.001**
Medium, *n* (%)	16,718 (40.1)	13,290 (40.5)	3,428 (38.7)	**<0.001**
High, *n* (%)	12,802 (30.7)	10,888 (33.1)	1,914 (21.6)	**<0.001**
Energy intake (kcal/day)	1,982 ± 647.4	1,973 ± 635.0	2,013.7 ± 635.0	**<0.001**
Smoking, *n* (%)	8,956 (21.0)	6,889 (20.5)	2,067 (22.8)	**<0.001**
Anthropometry				
BMI (kg/m^2)	25.9 ± 4.3	24.5 ± 2.9	31.4 ± 4.3	NA
Waist in men (cm)	95.4 ± 10.6	90.4 ± 7.2	105.6 ± 8.8	NA
Waist in women (cm)	86.9 ± 12.1	83.7 ± 8.9	106.1 ± 9.2	NA
Systolic BP (mm Hg)	125.7 ± 15.0	123.4 ± 14.2	133.8 ± 14.6	**<0.001**
Diastolic BP (mm Hg)	73.8 ± 9.1	72.7 ± 8.7	78.5 ± 9.3	**<0.001**
Lipids and inflammation				
Total cholesterol (mmol/L)	5.00 ± 0.98	4.90 ± 0.95	5.30 ± 1.02	**<0.001**
HDL-C in men (mmol/L)	1.2 (1.1-1.5)	1.3 (1.1-1.5)	1.1 (0.9-1.2)	**<0.001**
HDL-C in women (mmol/L)	1.5 (1.3-1.8)	1.6 (1.4-1.8)	1.3 (1.1-1.5)	**<0.001**
LDL-C (mmol/L)	3.17 ± 0.88	3.50 ± 0.91	3.89 ± 0.86	**<0.001**
Triglycerides (mmol/L)	1.0 (0.7-0.99)	0.9 (0.7-1.1)	1.6 (1.2-2.2)	NA
hsCRP (mg/L)	1.2 (0.6-2.8)	1.0 (0.5-2.2)	2.1 (1.1-4.8)	**<0.001**
Liver blood tests				
ALT (U/L)	19 (14-27)	18 (13-24)	28 (20-39)	**<0.001**
AST (U/L)	22 (19-27)	22 (19-26)	25 (21-30)	**<0.001**
ALP (U/L)	61.5 ± 17.0	59.4 ± 16.1	69.0 ± 17.7	**<0.001**
GGT (U/L)	20 (15-29)	18 (14-24)	33 (24-47)	NA
Glucose metabolism				
Plasma glucose (mmol/L)	5.0 ± 0.7	4.9 ± 0.6	5.4 ± 1.0	**<0.001**
HbA1c (%)	5.6 ± 0.4	5.5 ± 0.3	5.8 ± 0.6	**<0.001**
Glucose status: IGM, *n* (%)	14,444 (33.9)	10,154 (30.2)	4,290 (47.2)	**<0.001**
Glucose status: DM, *n* (%)	1,171 (2.7)	416 (1.2)	755 (8.3)	**<0.001**
Total MVPA				
No MVPA, *n* (%)	3,219 (7.5)	2,158 (6.4)	1,061 (11.7)	**<0.001**
MVPA (min/week)*	320 (120-795)	330 (140-786)	300 (90-840)	**<0.001**
Non-occupational MVPA				
No MVPA, *n* (%)	5,272 (12.4)	3,521 (10.5)	1,751 (19.3)	**<0.001**
MVPA (min/week)*	190 (60-360)	210 (90-380)	150 (30-330)	**<0.001**

Note: Data are presented as mean ± SD or median (25th to 75th percentile) and number (percentages). BMI, body mass index; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; IGM, impaired glucose metabolism; DM, diabetes mellitus; MVPA, moderate-to-vigorous physical activity; NA, not applicable.
*Adjusted for age, sex, and education level. NA: *P* values were not presented in the table because of the variables used in the FLI algorithm (BMI, waist, TG, and GGT).

Furthermore, participants with NAFLD had higher blood pressure and higher concentrations of total cholesterol, low-density lipoprotein cholesterol, FPG, HbA1c, and high-sensitivity C-reactive protein, as well as lower high-density lipoprotein cholesterol concentration, compared with subjects without NAFLD (all adjusted $p<0.001$). People with NAFLD were more likely to have IGM and T2DM. Other liver blood tests (e.g., ALT, AST, and ALP) were significantly associated with the presence of NAFLD. The adjusted means of total and non-occupational MVPA min/week were lower in the NAFLD group (**Figure S2**). Of all participants, 7.5% did not perform any activities at a moderate-to-vigorous level. Participant characteristics broken down by MVPA level are displayed in **Table S1**.

According to the results of logistic regression analysis, increased MVPA was associated with a low risk of NAFLD. The risk reduction associated with increased non-occupational MVPA was dose dependent. After further adjustment for daily caloric intake and smoking status, the associations were virtually the same, and dose dependency remained (**Table 2**). In the association between total MVPA and NAFLD, dose dependency disappeared at more active levels (MVPA-Q4 and Q5) when including the occupational MVPA (**Table 2, Figure S3**).

Table 2. Dose-dependent association between MVPA and NAFLD

MVPA categories	**Model 1**			**Model 2**		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Total daily-life MVPA:						
'No MVPA' (ref)	1.00	-	-	1.00	-	-
MVPA-Q1	0.68	0.61-0.76	**<0.001**	0.70	0.63-0.78	**<0.001**
MVPA-Q2	0.55	0.50-0.62	**<0.001**	0.57	0.51-0.64	**<0.001**
MVPA-Q3	0.48	0.43-0.53	**<0.001**	0.49	0.44-0.55	**<0.001**
MVPA-Q4	0.47	0.42-0.52	**<0.001**	0.49	0.44-0.55	**<0.001**
MVPA-Q5	0.55	0.49-0.61	**<0.001**	0.58	0.52-0.64	**<0.001**
Non-occupational MVPA:						
'No MVPA' (ref)	1.00	-	-	1.00	-	-
MVPA-Q1	0.77	0.70-0.84	**<0.001**	0.78	0.71-0.86	**<0.001**
MVPA-Q2	0.63	0.57-0.69	**<0.001**	0.64	0.58-0.70	**<0.001**
MVPA-Q3	0.52	0.47-0.58	**<0.001**	0.53	0.48-0.59	**<0.001**
MVPA-Q4	0.50	0.45-0.55	**<0.001**	0.51	0.46-0.56	**<0.001**
MVPA-Q5	0.44	0.40-0.49	**<0.001**	0.45	0.41-0.50	**<0.001**

Note: Binary logistic regression analysis. Reference group is the "No MVPA." Data are expressed as ORs and 95% confidence intervals (95% CIs). CI, confidence interval; MVPA, moderate-to-vigorous physical activity; OR, odds ratio; Q, quintile.

Model 1: adjusted for age, sex, and education.

Model 2: adjusted for age, sex, education, smoking, and daily caloric intake.

Furthermore, dose dependency seemed to be influenced by glucose status. At the highest level of MVPA (compared with No-MVPA), an OR (95% CI) of 0.49 (0.42; 0.57) was found for NGM, with values of 0.46 (0.40; 0.54) for IGM and 0.42 (0.27; 0.66) for T2DM (**Figure 1**).

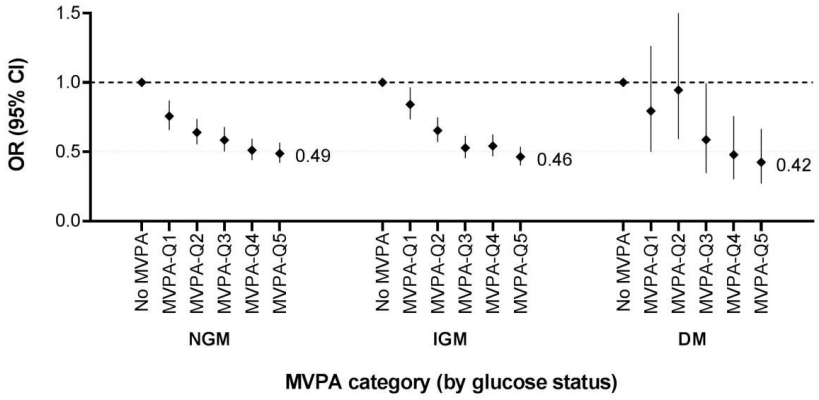

Figure 1. MVPA categories and the risk of having NAFLD by glucose status.

Note: Binary logistic regression analysis. Reference group is the "No MVPA." Data are expressed as OR and 95% CI. Error bars indicate 95% CIs. Analysis was adjusted for age, sex, education, smoking, and daily caloric intake. DM, diabetes mellitus; IGM, impaired glucose metabolism; MVPA, moderate-to-vigorous physical activity; NGM, normal glucose metabolism; OR, odds ratio; Q, quintile.

The association between MVPA and NAFLD was also dependent on age. The OR was 0.51 (0.42; 0.62) for adults aged 18-40 years, and it was reduced to 0.37 (0.29; 0.48) for adults aged 60-80 years, when comparing the highest level of MVPA with No-MVPA (**Figure 2**).

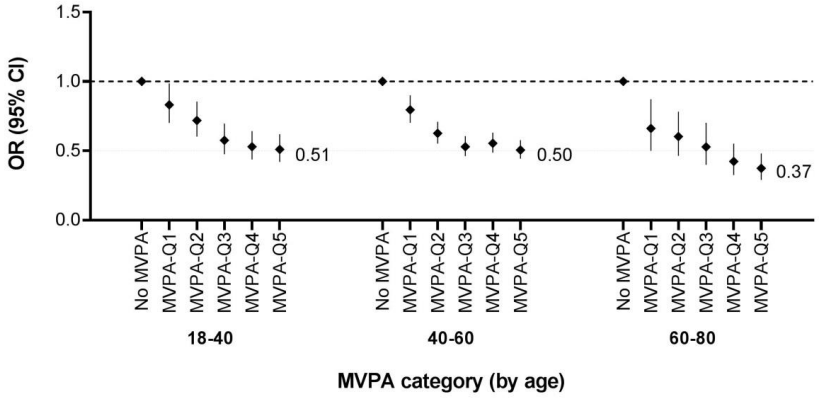

Figure 2. MVPA categories and the risk of having NAFLD by age.

Note: Binary logistic regression analysis. Reference group is the 'No MVPA'. Data are expressed as OR and 95% CI. Error bars indicate 95% CIs. Analysis was adjusted for age, sex, education, smoking, and daily caloric intake. MVPA, moderate-to-vigorous physical activity; OR, odds ratio; Q, quintile.

The results of linear regression analysis indicated that MVPA was inversely associated with the continuous measurement of the risk of NAFLD (Log-FLI) and its individual components (all $P<0.001$, **Tables 3**&**4**). These significant associations

were much stronger for TG and GGT than they were for BMI and WC (**Table 4**), thereby indicating that the association between MVPA and the FLI was mostly explained by the association between GGT and TG and not predominantly by the adiposity measures. Moreover, inverse associations were observed for other liver blood tests, including Log-ALT and Log-ALP (P<0.001, **Table 3**).

Table 3. Linear associations between MVPA and fatty liver biomarkers

MVPA	Unstandardized B (95% CI) ¶			
	FLI (score)	ALT (U/L)	AST (U/L)	ALP (U/L)
Total MVPA				
Overall	-0.038 (-0.044;-0.032)**	-0.006 (-0.009;-0.003)**	0.007 (0.005; 0.008)**	-0.006 (-0.008;-0.004)**
NGM	-0.027 (-0.035;-0.019)**	-0.004 (-0.008; 0.000)*	0.007 (0.005; 0.009)**	-0.005 (-0.007;-0.003)**
IGM	-0.049 (-0.059;-0.039)**	-0.008 (-0.012;-0.003)*	0.006 (0.003; 0.009)**	-0.006 (-0.009;-0.003)**
DM	-0.040 (-0.065;-0.016)*	-0.008 (-0.026;-0.010)	0.006 (-0.005; 0.018)	-0.008 (-0.016;0.004)
Non-occupational MVPA				
Overall	-0.061 (-0.066;-0.055)**	-0.009 (-0.008;-0.004)**	0.010 (0.008; 0.011)**	-0.006 (-0.008;-0.004)**
NGM	-0.046 (-0.054;-0.039)**	-0.005 (-0.009;-0.002)*	0.011 (0.009; 0.013)**	-0.004 (-0.006;-0.002)**
IGM	-0.073 (-0.082;-0.064)**	-0.011 (-0.016;-0.007)**	0.009 (0.006; 0.011)**	-0.008 (-0.011;-0.005)**
DM	-0.056 (-0.078;-0.034)**	-0.016 (-0.032;0.001)	-0.002 (-0.013; 0.009)	-0.006 (-0.015;0.003)
Occupational MVPA				
Overall	0.008 (0.001; 0.014)*	0.001 (-0.002; 0.003)	0.000 (-0.002; 0.002)	-0.002 (-0.004; 0.000)*

Note: Linear regression analysis. Data are expressed as unstandardized B and 95% confidence interval (95% CI). ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; DM, diabetes mellitus; FLI, fatty liver index; IGM, impaired glucose metabolism; MVPA, moderate-to-vigorous physical activity; NGM, normal glucose metabolism.¶ Adjusted for age, sex, education, smoking, and daily caloric intake. * P < 0.05. ** P < 0.001.

Table 4. Linear associations between MVPA and Individual components of fatty liver index

MVPA	Unstandardized B (95% CI) ¶			
	BMI (kg/m^2)	Waist (cm)	TG (mmol/L)	GGT (U/L)
Total MVPA				
Overall	-0.004 (-0.005;-0.003)**	-0.005 (-0.006;-0.004)**	-0.021 (-0.024;-0.017)**	-0.015 (-0.018;-0.012)**
NGM	-0.002 (-0.003;-0.001)*	-0.004 (-0.005;-0.003)**	-0.016 (-0.020;-0.012)**	-0.011 (-0.015;-0.006)**
IGM	-0.006 (-0.008;-0.004)**	-0.006 (-0.006;-0.004)**	-0.025 (-0.030;-0.019)**	-0.018 (-0.023;-0.012)**
DM	-0.008 (-0.015;-0.002)*	-0.007 (-0.012;-0.002)*	-0.035 (-0.056;-0.014)**	-0.031 (-0.052;-0.011)*
Non-occupational MVPA				
Overall	-0.009 (-0.010;-0.008)**	-0.009 (-0.010;-0.008)**	-0.022 (-0.025;-0.019)**	-0.017 (-0.02;-0.014)**
NGM	-0.006 (-0.007;-0.004)**	-0.007 (-0.008;-0.006)**	-0.018 (-0.022;-0.014)**	-0.010 (-0.014;-0.006)**
IGM	-0.011 (-0.012;-0.009)**	-0.011 (-0.012;-0.010)**	-0.026 (-0.031;-0.020)**	-0.021 (-0.026;-0.016)**
DM	-0.015 (-0.021;-0.009)**	-0.011 (-0.015;-0.007)**	-0.026 (-0.045;-0.007)*	-0.037 (-0.056;-0.019)**
Occupational MVPA				
Overall	0.003 (0.002; 0.004)**	0.002 (0.001; 0.003)**	-0.007 (-0.010;-0.004)**	-0.004 (-0.007; 0.000)*

Note: Linear regression analysis. Data are expressed as unstandardized B and 95% confidence interval (95% CI). BMI, body mass index; CI, confidence interval; DM, diabetes mellitus; GGT, gamma-glutamyltransferase; IGM, impaired glucose metabolism; MVPA, moderate-to-vigorous physical activity; NGM, normal glucose metabolism; TG, triglycerides.¶ Adjusted for age, sex, education, smoking, and daily caloric intake. * P < 0.05. ** P < 0.001.

A positive association was found between MVPA and Log-AST (P<0.001). Occupational MVPA was positively associated with Log-FLI, Log-BMI and Log-WC, and it was inversely associated with Log-TG, Log-GGT and Log-ALP (P<0.001),

although the β-coefficients were small (**Tables 3**&**4**). Higher MVPA was significantly associated with lower NFS, but positively associated with FIB-4 and APRI (**Table 5**).

Table 5. Linear associations between MVPA and fibrosis in NAFLD

MVPA	FIB-4		APRI		NFS	
	B (95% CI)	*P*	B (95% CI)	*P*	B (95% CI)	*P*
MVPA-Q1 *vs* No-MVPA	0.010 (-0.01;0.03)	0.363	0.017 (-0.01;0.043)	0.215	-0.037 (-0.01-0.04)	0.342
MVPA-Q2 *vs* No-MVPA	0.010 (-0.01;0.02)	0.076	0.013 (0.001;0.03)	0.051	-0.014 (-0.05-0.03)	0.471
MVPA-Q3 *vs* No-MVPA	0.010 (0.002;0.02)	0.011	0.012 0.002;0.022)	0.015	-0.029 (-0.06-0.00)	0.087
MVPA-Q4 *vs* No-MVPA	0.005 (-0.01;0.01)	0.900	0.008 0.001;0.015)	0.037	-0.027 (-0.05;-0.01)	0.047
MVPA-Q5 *vs* No-MVPA	0.043 (0.02;0.066)	0.001	0.044 0.015;0.072)	0.003	-0.030 (-0.11;-0.01)	0.011

Note: Linear regression analysis. Data are expressed as unstandardized B and 95% confidence interval (95% CI) indicating the associations of each MVPA level compared with the category of No MVPA. Levels of MVPA are used as "dummy' variables. Analysis was adjusted for age, sex, education, smoking, and daily caloric intake. APRI, AST-to-platelet ratio index; CI, confidence interval; FIB-4, fibrosis-4; MVPA, moderate-to-vigorous physical activity; NFS, NAFLD Fibrosis Score.

FIB-4 Score = (Age*AST) / (Platelets*√(ALT)).

APRI = (AST in IU/L) / (AST Upper Limit of Normal in IU/L) / (Platelets in 10^9/L).

NAFLD-Fibrosis Score = -1.675 + (0.037*age [years]) + (0.094*BMI [kg/m^2]) + (1.13*IFG/diabetes [yes = 1, no = 0]) + (0.99*AST/ALT ratio) – (0.013*platelet count [×10^9/L]) – (0.66*albumin [g/dl]).

Sensitivity analysis revealed that being inactive (No-MVPA) increased the risk of NAFLD by an OR of 1.43 (1.29;1.60) for total MVPA and 1.28 (1.67;1.41) for non-occupational MVPA, as compared with being "a little active" (MVPA-Q1) (**Figure S3**). Furthermore, the dose-dependent association was confirmed using the time spent engaging in sports as a determinant of the risk of NAFLD (**Table S3**). Further sensitivity analysis revealed dose dependent associations between MVPA and NAFLD across all categories of alcohol consumption, including for the excessive alcohol users who had been excluded from the main analysis (**Table S4**).

DISCUSSION

This large-scale population-based study makes a substantial contribution to the existing evidence on the potential benefits of increased physical activity on NAFLD. We established a dose-response relationship between daily-life physical activity and the risk of having NAFLD, demonstrating that more physical activity is more beneficial. If occupational MVPA is included in the level of total physical activity, however, individuals who are much more active may not experience any additional benefit. These results indicate that the potentially beneficial effects of physical activity are dependent on particular types of daily-life activity. Extreme levels of occupational physical activity are not protective for NAFLD. The potentially beneficial effects of physical activity apply to all other activities at the moderate-to-vigorous level (e.g., commuting, leisure time, or sport). In general, older individuals and

individuals with IGM or T2DM experience larger reductions in the risk of having NAFLD, relative to younger and healthier individuals.

In line with our results, a few earlier studies have established that increased levels of daily-life physical activity are associated with a reduction in the incidence of NAFLD. For example, Perseghin et al. demonstrated that the prevalence of NAFLD was lower for most physically active individuals [8]. Kwak et al. reported a similar inverse association between daily-life physical activities and the risk of NAFLD [9]. Kistler et al. found a dose-dependent association between time spent on MVPA and biopsy-proven NAFLD scores [13]. Results of a larger meta-analysis were nevertheless inconsistent with regard to the dose-dependent association between MVPA and NAFLD [5]. The study did not detect any dose-dependency related to time spent exercising. This result may have been due to either a lack of statistical power because of small sample sizes, or a limitation of individual data analysis from the trials. In an individual trial by Oh et al., however, extensive time spent in MVPA ($\geqslant$250 min/week) had a greater beneficial effect in the pathophysiology of NAFLD than did shorter periods of activity (<150 min/week)[30]. Finally, our large population based study provides evidence of a dose-dependent association between time spent on MVPA and the risk of having NAFLD.

As demonstrated by our results, a transition from the least active level to each increasing level of MVPA could be beneficial in terms of NAFLD. Even an activity level lower than the recommendation (>150min/week) i.e., the lowest level of MVPA ('MVPA-Q1') was better than being entirely inactive (No-MVPA). Our results suggest that people whose activity is at the recommended level (150-200 min/week)[1] or higher are at lower risk of having NAFLD. If occupational activities are taken into account, however, levels of activity that greatly exceed the guidelines (MVPA-Q4 and Q5) might not generate any additional benefits. This result might be due to the inclusion of occupational activity, which may not offer the same direct health benefits that are associated with leisure time physical activity.

The finding that occupational MVPA offers no clear health benefit is in line with results from other studies [31-33]. For example, a meta-analysis indicated that OPA is not beneficial in terms of protection against hypertension [31]. In other studies, Larsson et al. reported a positive association between OPA and insulin resistance [32] and Lund et al. identified a longitudinal association between heavy occupational activity and sickness absence[33]. The mechanism that apparently prevents occupational physical activity from generating additional health benefits is unclear. Of course, there may be the possibility of confounding, that normally overweight participants are both inactive and have a higher risk for NAFLD. For such individuals, the barriers against exercise may only be overcome in the context of occupational activities, thus generating an association between high occupational MVPA and a high NAFLD risk. On the other hand, exercise interventions do seem to lower the

level of liver fat, and several biological mechanisms have been suggested. Biological explanations might be related to the type of activity (e.g., heavy lifting or pushing and extreme bending or twisting of the neck or back without longer periods of rest for recovery) [33]. Astrand et al. identified an association between work-based activities (e.g., working with hands above shoulder level) and increased blood pressure [34]. The types of occupation related to high occupational MVPA in our study included such occupations as "metal, machinery, and related trade work," "handicraft and printing work," and "other mechanics and repairs". Although the association between occupational MVPA and health cannot be fully explained, it is important to be aware that occupational MVPA should not be considered as a substitute for leisure time MVPA.

In our study, the association between MVPA and NAFLD was stronger for older ages. One possible explanation for this result might be that benefits are gained more easily when there is more room for improvement (as is the case for older people). The young people in this study were healthy, irrespective of their lifestyles. In accordance with our results, several studies have identified that lifestyle interventions (including physical activity) had greater benefits for the oldest individuals [35-36]. Results from a prevention program demonstrated an inverse relationship between age and the incidence of diabetes among participants, compared with a control group [35]. In the Finnish Diabetes Prevention Study, intervention was more effective in the oldest tertile of the population [36].

In line with previous studies, the prevalence of NAFLD was higher in individuals with T2DM in our study [16-18]. This could be because the risk of NAFLD is strongly interrelated with the risk of T2DM, insulin resistance, and the metabolic syndrome [37-41]. With regard to the association between MVPA and the risk of NAFLD, the magnitude of the effect was greater in people with diabetes than it was in the NGM and IGM groups in our study. As was the case with older age, one explanation for this result could be that benefits are gained more easily when there is more room for improvement. Accordingly, if people manage to remain more active despite their diabetes, they are more likely to remain relatively healthy.

Concerns could be expressed about using the FLI to identify individuals with NAFLD. Studies have indicated that the clinical utility of the FLI is limited, largely because it fails to correctly distinguish between moderate and severe steatosis [42-43]. Nevertheless, the FLI has revealed a linear trend across steatosis grades, as classified by histology in liver biopsies [43]. The study showed that the Area Under the curve of the Receiver Operating Characteristic (AUROC) value for the FLI was 0.83, indicating good diagnostic accuracy for the presence or absence of NAFLD. Given that the latter criterion was the most important outcome in our study, and given that we did not consider the severity of NALFD, the use of the FLI could not have caused serious classification bias in this study. Further development of

appropriate and accurate quantitative markers for NAFLD would be very useful for both clinical use and research purposes.

In our study, we also assessed the impact of MVPA on other liver blood tests and fibrosis makers. The inverse associations that we found for ALT, ALP, and GGT provide evidence of a relationship between daily-life MVPA and liver health. However, we also found a positive association with AST, which could offer a partial explanation for the positive associations between MVPA and FIB-4 or APRI. On closer inspection, this result could have been due to the fact that FIB-4 and APRI were based on fewer parameters than NFS was, in addition to being largely dependent on AST. Because AST may also originate from skeletal muscle, a positive association between MVPA and AST could be explained by the increased breakdown of muscle cells with increasing physical activity, thereby resulting in a higher concentration of AST [44]. On the basis of another FB score (the NFS), however, higher levels of MVPA seem to be related to a lower risk of FB. The association between MVPA and FB markers is thus inconclusive. It is nevertheless important to consider the possibility that FIB4 and APRI are not suitable as makers for research on the role of physical activity and liver health.

The greatest strength of our study is that it is based on a large sample from the general population, thereby allowing us to estimate the dose dependency of MVPA with regard to NAFLD in various subgroups (e.g., different levels of glucose status, different age groups) with sufficient statistical power. The study is nevertheless subject to several limitations as well. One is related to the use of the FLI to identify NAFLD. Although the FLI does not provide an absolute measure of the accumulation of fat in the liver, it is one of the best-validated markers for steatosis, especially in large-scale screening [1, 45]. Another limitation has to do with our assessment of physical activity and information about hepatitis and cirrhosis based on self-reports. It should be noted that some subjects might have undiagnosed viral hepatitis. Finally, our study design was cross-sectional.

CONCLUSIONS

A higher level of non-occupational daily-life moderate-to-vigorous physical activity is dose-dependently associated with a lower risk of having NAFLD, based on a non-invasive marker for the risk of fatty liver. With regard to the level of physical activity, any increase in MVPA, even at levels lower than those recommended by the relevant guidelines, is still better than being entirely inactive. The risk is further reduced for individuals who are more active than recommended. If occupational MVPA is included in the level of total daily-life MVPA, however, individuals who are much more active than the guidelines recommend may not obtain any additional benefit. Nevertheless,

our results indicate that increased physical activity is accompanied by a lower risk of having NAFLD, although extreme levels of occupational MVPA are not protective. The association between non-occupational MVPA and the risk of having NAFLD is stronger in people with diabetes and older adults, suggesting that people who manage to remain active will be the healthiest.

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CONFLICT OF INTEREST

Guarantors of the article: Eva Corpeleijn, PhD
Specific author contributions: Oyuntugs Byambasukh analyzed the data, designed the study's analytic strategy, and interpreted the results. Dorien Zelle contributed to the hypothesis and edited the manuscript. Eva Corpeleijn planned and designed the study, analyzed the data, directed its implementation, and reviewed the manuscript. All authors contributed to the critical revision of the manuscript.
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STUDY HIGHLIGHTS

What is current knowledge

- ✓ The risk of NAFLD is lower for people with higher levels of physical activity.
- ✓ Large population-based studies describing the association of MVPA and NAFLD across domains of daily-life physical activity, age groups, and glucose status are lacking.

What is new here

- ✓ Higher levels of physical activity are associated with lower risk of NAFLD, although extreme levels of occupational physical activity are not protective.

- ✓ Older individuals experience a relatively larger reduction in NAFLD risk from physical activity than do younger adults.
- ✓ Individuals with diabetes experience a larger reduction in NAFLD risk from physical activity than do healthy adults.

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SUPPLEMENTARY MATERIALS

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Supplementary methods: Liver fibrosis markers

Non-invasive markers for liver fibrosis were used to define risk of fibrosis in this study, as follows:

1. Fibrosis 4 Score (FIB-4) = (Age*AST) / (Platelets*√(ALT)), where age in years, AST in IU/L, platelets in 10^9/L and ALT in IU/L.
2. AST to Platelet Ratio Index (APRI) = (AST in IU/L) / (AST Upper Limit of Normal in IU/L) / (Platelets in 10^9/L).
3. NAFLD-Fibrosis Score (NFS) = -1.675 + (0.037*age [years]) + (0.094*BMI [kg/m^2]) + (1.13*IFG/diabetes [yes = 1, no = 0]) + (0.99*AST/ALT ratio) – (0.013*platelet count [$\times 10^9$/L]) – (0.66*albumin [g/dl]).

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Table S1A. Characteristics of the study population, according to total daily-life MVPA categories

Variable	MVPA category					
	No-MVPA	MVPA-Q1	MVPA-Q2	MVPA-Q3	MVPA-Q4	MVPA-Q5
N (%)	3,219 (7.5)	7,991 (18.7)	7,482 (17.5)	8,402 (19.7)	7,678 (18.0)	7,889 (18.5)
Total MVPA (min/week)	0	1-135	136-269	270-480	481-1105	1106-6840
Non-occupational MVPA*	0	14.7 (6.3-23.1)	25.9 (17.2-34.7)	50.3 (42.0-58.7)	257.7 (249.1-266.4)	1533.9 (1525.2-1542.6)
Occupational MVPA*	0	70.8 (65.5-76.2)	181.5 (175.9-187.0)	317.7 (312.5-322.9)	470.9 (465.6-476.4)	429.7 (424.2-435.2)
Age (years)	46 (38-52)¶	44 (37-51)† ¶	44 (36-51)† ¶	45 (36-53)† ¶	45 (36-52)† ¶	42 (34-49)†
Male sex, *n* (%)	1,366 (42.4)¶	2,773 (34.7)† ¶	2,588 (34.6)† ¶	3,142 (37.4)† ¶	2,974 (38.7)† ¶	4,028 (51.1)†
Education: Low, *n* (%)	631 (19.6)¶	985 (12.3)† ¶	861 (11.5)† ¶	1,042 (12.4)† ¶	1,220 (15.9)† ¶	1,678 (21.3)†
Energy intake (kcal/day)	1882.8 ± 565.1¶	1912.6 ± 541.0† ¶	1917.2 ± 530.2† ¶	1955.4 ± 563.5† ¶	2003.0 ± 608.3† ¶	2167.8 ± 736.1†
Smoking, *n* (%)	1,043 (32.4)¶	1,716 (21.5)†	1,314 (17.6)† ¶	1,378 (16.4)† ¶	1,468 (19.1)† ¶	2,037 (25.8)†
BMI (kg/m2)	27.1 ± 4.9¶	26.1 ± 4.4†	25.7 ± 4.2† ¶	25.7 ± 4.1† ¶	25.8 ± 4.3† ¶	26.2 ± 4.3†
Waist in men (cm)	99.3 ± 11.6¶	96.5 ± 10.4†	95.1 ± 10.2† ¶	94.4 ± 10.1† ¶	94.0 ± 10.5† ¶	95.0 ± 10.6†
Waist in women (cm)	90.0 ± 13.1¶	87.6 ± 12.1†	86.4 ± 11.9† ¶	86.1 ± 11.8† ¶	86.4 ± 11.9† ¶	87.1 ± 12.5†
Systolic BP (mm Hg)	128.2 ± 15.4¶	125.7 ± 15.2†	125.0 ± 15.0† ¶	125.2 ± 15.2† ¶	125.3 ± 14.9† ¶	126.3 ± 14.3†
Total cholesterol (mmol/L)	5.07 ± 1.00¶	5.01 ± 0.98	5.01 ± 0.98 ¶	5.00 ± 0.99† ¶	4.97 ± 0.99† ¶	4.99 ± 0.95†
HDL (mmol/L) in men	1.10 (1.0-1.3)¶	1.20 (1.0-1.4)†	1.20 (1.1-1.4)†¶	1.30 (1.1-1.5)†¶	1.30 (1.1-1.5)†¶	1.30 (1.1-1.5)†
HDL (mmol/L) in women	1.50 (1.2-1.7)¶	1.50 (1.3-1.8)†	1.60 (1.3-1.8)† ¶	1.60 (1.3-1.8)† ¶	1.60 (1.3-1.9)† ¶	1.50 (1.3-1.8)†
Triglycerides (mmol/L)	1.12 (0.8-1.63)¶	1.01 (0.7-1.44)†	0.97 (0.7-1.4)†¶	0.96 (0.7-1.4)†¶	0.96 (0.7-1.3)†¶	0.98 (0.7-1.42)†
Plasma glucose (mmol/L)	5.13 ± 0.89¶	5.00 ± 0.76†	4.97 ± 0.72† ¶	4.98 ± 0.74† ¶	4.97 ± 0.69† ¶	5.01 ± 0.65†
ALT (U/L)	20.0 (14-30)¶	19.0 (14-27)†	19.0 (14-27)† ¶	19.0 (14-26)† ¶	19.0 (14-26)† ¶	20.0 (15-28)†
AST (U/L)	22.0 (19-26)¶	22.0 (19-26)† ¶	22.0 (19-26)† ¶	23.0 (19-27)† ¶	23.0 (19-27)† ¶	23.0 (20-27)†
ALP (U/L)	64.7 ± 18.2¶	61.8 ± 18.0†	60.5 ± 16.3† ¶	60.8 ± 16.5† ¶	61.2 ± 16.7† ¶	61.6 ± 16.5†
GGT (U/L)	23.0 (16-34)¶	20.0 (15-29)†	20.0 (15-28)† ¶	20.0 (14-28)† ¶	19.0 (15-27)† ¶	21.0 (15-30)†
NAFLD, *n* (%)	1061 (33.0)¶	1811 (22.7)†	1437 (19.2)† ¶	1526 (18.2)† ¶	1422 (18.5)† ¶	1824 (23.1)†
IGM, *n* (%)	1,258 (39.1)¶	2,688 (33.6)†	2,411 (32.2)† ¶	2,846 (33.9)† ¶	2,526 (32.9)† ¶	2,715 (34.4)†
Diabetes, *n* (%)	164 (5.1)¶	240 (3.0)†	182 (2.4)† ¶	222 (2.6)† ¶	214 (2.8)† ¶	149 (1.9)†

Note: Data are presented as mean ± SD or median (25^{th} to 75^{th} percentile) and number (percentages, %). *Adjusted for age, sex and education. MVPA, moderate-to-vigorous activity level; BMI, body mass index; BP, blood pressure; HDL, high density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, Alkaline phosphatase; GGT, gamma-glutamyltransferase; NAFLD, Non-alcoholic fatty liver disease; IGM, impaired glucose metabolism. † P<0.05 vs. No-MVPA; ¶ P<0.05 vs. MVPA-Q5. ‡ P<0.001 vs. Between groups. Significance tested using Bonferroni post hoc analysis and Pearson Chi-Square test.

Table S1B. Characteristics of the study population, according to non-occupational MVPA categories

Variable	MVPA category					
	No-MVPA	MVPA-Q1	MVPA-Q2	MVPA-Q3	MVPA-Q4	MVPA-Q5
N (%)	5,272 (12.4)	7,527 (17.6)	8,248 (19.3)	6,650 (15.6)	7,479 (17.5)	7,485 (17.5)
Non-occupational MVPA (min/week)‡	0	1-90	91-180	181-292	293-464	465-1150
Age (years)	45 (37-50)	43 (36-50)†	44 (36-50)	44 (36-50)†	44 (35-52)	46 (37-55)†
Male sex, *n* (%)	2,654 (50.3)	2,837 (37.7)†	3,022 (36.6)†	2,327 (35.0)†	2,825 (37.8)†	3,206 (42.8)†
Education: Low, *n* (%)	1,201 (22.8)	1,091 (14.5)†	1,112 (13.5)†	797 (12.0)†	1,012 (13.5)†	1,204 (16.1)†
Energy intake (kcal/day)	2002.8 ± 655.1	1981.7 ± 535.4	1953.4 ± 574.8†	1957.1 ± 576.8†	1983.0 ± 611.3	2021.9 ± 632.8
Smoking, *n* (%)	1,823 (34.6)	1,798 (23.9)†	1,698 (20.6)†	1,178 (17.7)†	1,237 (16.5)†	1,222 (16.3)†
BMI (kg/m2)	29.9 ± 4.8	26.2 ± 4.5†	25.9 ± 4.3†	25.6 ± 4.2†	25.7 ± 4.1†	25.7 ± 4.0†
Waist in men (cm)	98.3 ± 11.5	96.4 ± 10.7†	95.7 ± 10.0†	94.0 ± 9.9†	94.0 ± 10.1†	93.5 ± 10.3†
Waist in women (cm)	90.0 ± 13.3	88.0 ± 12.4†	87.0 ± 12.0†	86.0 ± 11.8†	86.0 ± 11.7†	85.8 ± 11.7†
Systolic BP (mm Hg)	128.4 ± 14.9	125.7 ± 14.7†	125.3 ± 14.9†	124.5 ± 14.7†	125.1 ± 15.1†	128.7 ± 15.1†
Total cholesterol (mmol/L)	5.07 ± 1.00	5.01 ± 0.98†	5.01 ± 0.98†	5.00 ± 0.99†	4.97 ± 0.99†	4.99 ± 0.95
HDL (mmol/L) in men	1.20 (1.0-1.3)	1.20 (1.0-1.4)†	1.20 (1.1-1.4)†	1.30 (1.1-1.5)†	1.30 (1.1-1.5)†	1.30 (1.1-1.5)†
HDL (mmol/L) in women	1.40 (1.2-1.7)	1.50 (1.3-1.8)†	1.50 (1.3-1.8)†	1.60 (1.3-1.8)†	1.60 (1.3-1.9)†	1.60 (1.4-1.9)†
Triglycerides (mmol/L)	1.11 (0.80-1.63)	1.02 (0.73-1.45)†	1.00 (0.72-1.41)†	0.94 (0.70-1.32)†	0.95 (0.70-1.33)†	0.95 (0.70-1.33)†
Plasma glucose (mmol/L)	5.11 ± 0.81	5.00 ± 0.76†	4.99 ± 0.73†	4.94 ± 0.65†	4.98 ± 0.72†	5.01 ± 0.77†
ALT (U/L)	21.0 (15-21)	19.0 (14-27)†	19.0 (14-27)†	19.0 (14-26)†	19.0 (14-26)†	20.0 (15-28)†
AST (U/L)	22.0 (19-27)	22.0 (19-26)†	22.0 (19-26)	22.0 (19-26)	23.0 (20-27)†	23.0 (20-28)†
ALP (U/L)	64.6 ± 17.8	62.0 ± 18.1†	60.3 ± 16.6†	60.3 ± 17.2†	60.8 ± 16.5†	61.0 ± 16.5†
GGT (U/L)	23.0 (16-34)	20.0 (15-30)†	20.0 (15-29)†	19.0 (14-27)†	19.0 (15-28)†	20.0 (15-28)†
NAFLD, *n* (%)	1,751 (33.2)	1,803 (24.0)†	1,714 (20.8)†	1,150 (17.3)†	1,320 (17.6)†	1,343 (17.9)†
IGM, *n* (%)	2,041 (38.7)	2,522 (33.5)†	2,736 (33.2)†	2,127 (32.0)†	2,461 (32.9)†	2,557 (34.2)†
Diabetes, *n* (%)	213 (4.0)	215 (2.9)†	222 (2.7)†	121 (1.8)†	187 (2.5)†	213 (2.8)†

Note: Data are presented as mean ± SD or median (25^{th} to 75^{th} percentile) and number (percentages, %). *Adjusted for age, sex and education. MVPA, moderate-to-vigorous activity level; BMI, body mass index; BP, blood pressure; HDL, high density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, Alkaline phosphatase; GGT, gamma-glutamyltransferase; NAFLD, Non-alcoholic fatty liver disease; IGM, impaired glucose metabolism. † P<0.05 vs. No-MVPA; ¶ P<0.05 vs. MVPA-Q5. ‡ P<0.001 vs. Between groups. Significance tested using Bonferroni post hoc analysis and Pearson Chi-Square test.

Table S2. Linear associations between MVPA and fatty liver biomarkers, according to sex, age and education

MVPA	Unstandardized B (95% CI) ¶			
	Fatty liver biomarkers			
	FLI (score)	ALT (U/L)	AST (U/L)	ALP (U/L)
Sex				
Male	-0.060 (-0.068;-0.053)**	-0.023 (-0.027;-0.019)**	0.008 (0.005;0.010)**	-0.003 (-0.006;-0.001)*
Female	-0.062 (-0.071;-0.054)**	0.002 (-0.002;0.005)	0.011 (0.009;0.013)**	-0.009 (-0.011;-0.006)**
Education				
Low	-0.051 (-0.064;-0.038)**	-0.004 (-0.011;0.003)*	0.009 (0.005;0.013)*	-0.004 (-0.008;0.000)**
Medium	-0.058 (-0.067;-0.050)**	-0.010 (-0.014;-0.006)**	0.008 (0.006;0.010)**	-0.006 (-0.008;-0.003)**
High	-0.068 (-0.077;-0.058)**	-0.008 (-0.013;-0.004)**	0.011 (0.009;0.014)**	-0.008 (-0.011;-0.005)**
Age				
<40	-0.049 (-0.059;-0.038)**	-0.007 (-0.011;-0.002)**	0.009 (0.007;0.012)**	-0.007 (-0.010;-0.004)**
40-60	-0.058 (-0.066;-0.050)**	-0.007(-0.011;-0.003)**	0.010 (0.007;0.012)**	-0.009 (-0.011;-0.006)**
>60	-0.074 (-0.088;-0.060)**	-0.012 (-0.019;-0.005)*	0.003 (-0.001;0.007)*	-0.008 (-0.013;-0.004)**
MVPA	**Individual components of FLI**			
	BMI (kg/m^2)	Waist (cm)	TG (mmol/L)	GGT (U/L)
Sex				
Male	-0.007 (-0.009;-0.006)**	-0.009 (-0.010;-0.008)**	-0.031 (-0.036;-0.026)**	-0.027 (-0.032;-0.023)**
Female	-0.010 (-0.011;-0.008)**	-0.009 (-0.010;-0.008)**	-0.016 (-0.020;-0.012)**	-0.008 (-0.012;-0.005)**
Education				
Low	-0.009 (-0.012;-0.007)**	-0.009 (-0.011;-0.007)**	-0.019 (-0.027;-0.011)**	-0.015 (-0.023;-0.008)**
Medium	-0.008 (-0.009;-0.006)**	-0.009 (-0.010;-0.008)**	-0.023 (-0.028;-0.018)**	-0.018 (-0.022;-0.013)**
High	-0.009 -0.011;-0.008)**	-0.010 (-0.011;-0.009)**	-0.024 (-0.029;-0.019)**	-0.015 (-0.020;-0.011)**
Age				
<40	-0.005 (-0.007;-0.004)**	-0.007 (-0.008;-0.006)**	-0.023 (-0.028;-0.018)**	-0.012(-0.017;-0.007)**
40-60	-0.008 (-0.009;-0.007)**	-0.009 (-0.010;-0.008)**	-0.022 (-0.026;-0.017)**	-0.017 (-0.021;-0.012)**
>60	-0.012 (-0.015;-0.010)**	-0.011 (-0.013;-0.009)**	-0.026 (-0.034;-0.018)**	-0.026 (-0.034;-0.017)**

Note: Linear regression analysis. Data are expressed as unstandardized B and 95% confidence interval (95% CI). MVPA, moderate-to-vigorous physical activity; FLI, fatty liver index; ALT alanine aminotransferase; AST, aspartate aminotransferase; ALP, Alkaline phosphatase,; BMI, body mass index; TG, triglycerides; GGT, gamma-glutamyl transferase; NGM, normal glucose metabolism; IGM, impaired glucose metabolism; DM, diabetes mellitus. ¶ adjusted for age, sex, education, smoking and daily caloric intake. *P<0.05, **P<0.001.

Table S3. Sensitivity analysis for dose-dependent association between MVPA and NAFLD

MVPA	Model 1			Model 2		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Overall						
'No MVPA Sport' (ref)	1.0			1.0		
MVPA-Q1 Sport	0.68	0.62-0.74	**<0.001**	0.69	0.63-0.74	**<0.001**
MVPA-Q2 Sport	0.68	0.61-0.77	**<0.001**	0.69	0.61-0.78	**<0.001**
MVPA-Q3 Sport	0.69	0.63-0.76	**<0.001**	0.69	0.63-0.77	**<0.001**
MVPA-Q4 Sport	0.65	0.59-0.71	**<0.001**	0.65	0.59-0.72	**<0.001**
MVPA-Q5 Sport	0.51	0.46-0.56	**<0.001**	0.51	0.46-0.57	**<0.001**

Note: Binary logistic regression analysis. Reference group is the 'No MVPA'. Data are expressed as odds ratios (OR) and 95% confidence interval (95% CI). Model1: Adjusted for age, sex and education. Model2: Adjusted for age, sex and education, smoking and daily caloric intake.

Table S3. *(continued).*

MVPA	Model 1			Model 2		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
NGM						
'No MVPA Sport' (ref)	1.0			1.0		
MVPA-Q1 Sport	0.65	0.56-0.74	**<0.001**	0.66	0.57-0.76	**<0.001**
MVPA-Q2 Sport	0.71	0.59-0.84	**<0.001**	0.72	0.60-0.86	**<0.001**
MVPA-Q3 Sport	0.72	0.62-0.83	**<0.001**	0.73	0.63-0.84	**<0.001**
MVPA-Q4 Sport	0.67	0.58-0.77	**<0.001**	0.68	0.59-0.78	**<0.001**
MVPA-Q5 Sport	0.57	0.49-0.66	**<0.001**	0.58	0.50-0.67	**<0.001**
IGM						
'No MVPA Sport' (ref)	1.0			1.0		
MVPA-Q1 Sport	0.75	0.66-0.86	**<0.001**	0.75	0.66-0.86	**<0.001**
MVPA-Q2 Sport	0.66	0.57-0.79	**<0.001**	0.67	0.56-0.80	**<0.001**
MVPA-Q3 Sport	0.66	0.57-0.76	**<0.001**	0.65	0.56-0.76	**<0.001**
MVPA-Q4 Sport	0.65	0.57-0.75	**<0.001**	0.64	0.56-0.74	**<0.001**
MVPA-Q5 Sport	0.50	0.42-0.58	**<0.001**	0.49	0.42-0.58	**<0.001**
DM						
'No MVPA Sport' (ref)	1.0			1.0		
MVPA-Q1 Sport	0.64	0.42-0.99	**0.044**	0.63	0.41-0.98	**0.040**
MVPA-Q2 Sport	0.89	0.47-1.68	0.073	0.86	0.45-1.62	0.063
MVPA-Q3 Sport	0.87	0.55-1.38	0.054	0.86	0.54-1.37	0.052
MVPA-Q4 Sport	0.60	0.38-0.95	0.003	0.58	0.37-0.92	0.019
MVPA-Q5 Sport	0.34	0.19-0.60	**<0.001**	0.32	0.18-0.56	**<0.001**

Table S4. Association between MVPA and NAFLD by alcohol consumption

Alcohol categories	MVPA categories	Risk of NAFLD		
		OR	95% CI	*P-value*
Tertile 1	'No MVPA' (ref)	1.00	-	-
(0-1.6)*	MVPA-Q1	0,74	0.64-0.86	**<0.001**
n=10,991	MVPA-Q2	0,63	0.54-0.74	**<0.001**
	MVPA-Q3	0,52	0.44-0.62	**<0.001**
	MVPA-Q4	0,57	0.48-0.67	**<0.001**
	MVPA-Q5	0,49	0.41-0.57	**<0.001**
Tertile 2	'No MVPA' (ref)	1.00	-	-
(1.61-6.71)*	MVPA-Q1	0,80	0.67-0.95	**0.012**
n=10,943	MVPA-Q2	0,68	0.57-0.81	**<0.001**
	MVPA-Q3	0,59	0.49-0.71	**<0.001**
	MVPA-Q4	0,48	0.40-0.58	**<0.001**
	MVPA-Q5	0,45	0.37-0.54	**<0.001**
Tertile 3	'No MVPA' (ref)	1.00	-	-
(6.72-27.9)*	MVPA-Q1	0,83	0.70-0.97	**0.023**
n=11,049	MVPA-Q2	0,63	0.54-0.75	**<.001**
	MVPA-Q3	0,52	0.44-0.63	**<0.001**
	MVPA-Q4	0,51	0.43-0.60	**<0.001**
	MVPA-Q5	0,46	0.39-0.54	**<0.001**

Note: Binary logistic regression analysis. Reference group is the 'No MVPA'. Data are expressed as odds ratios (OR) and 95% confidence interval (95% CI). Analysis was adjusted for age, sex, education, smoking and daily caloric intake. * Alcohol intake (g/day) was expressed as minimum-maximum.

Table S4. *(continued).*

Alcohol categories	MVPA categories	Risk of NAFLD		
		OR	95% CI	*P-value*
Excessive users (20-107.8)* n=2,908	'No MVPA' (ref)	1.00	-	-
	MVPA-Q1	0,91	0.65-1.19	0.589
	MVPA-Q2	0,77	0.54-1.12	0.153
	MVPA-Q3	0,90	0.60-1.34	0.595
	MVPA-Q4	0,59	0.40-0.86	**0.006**
	MVPA-Q5	0,64	0.45-0.93	**0.019**

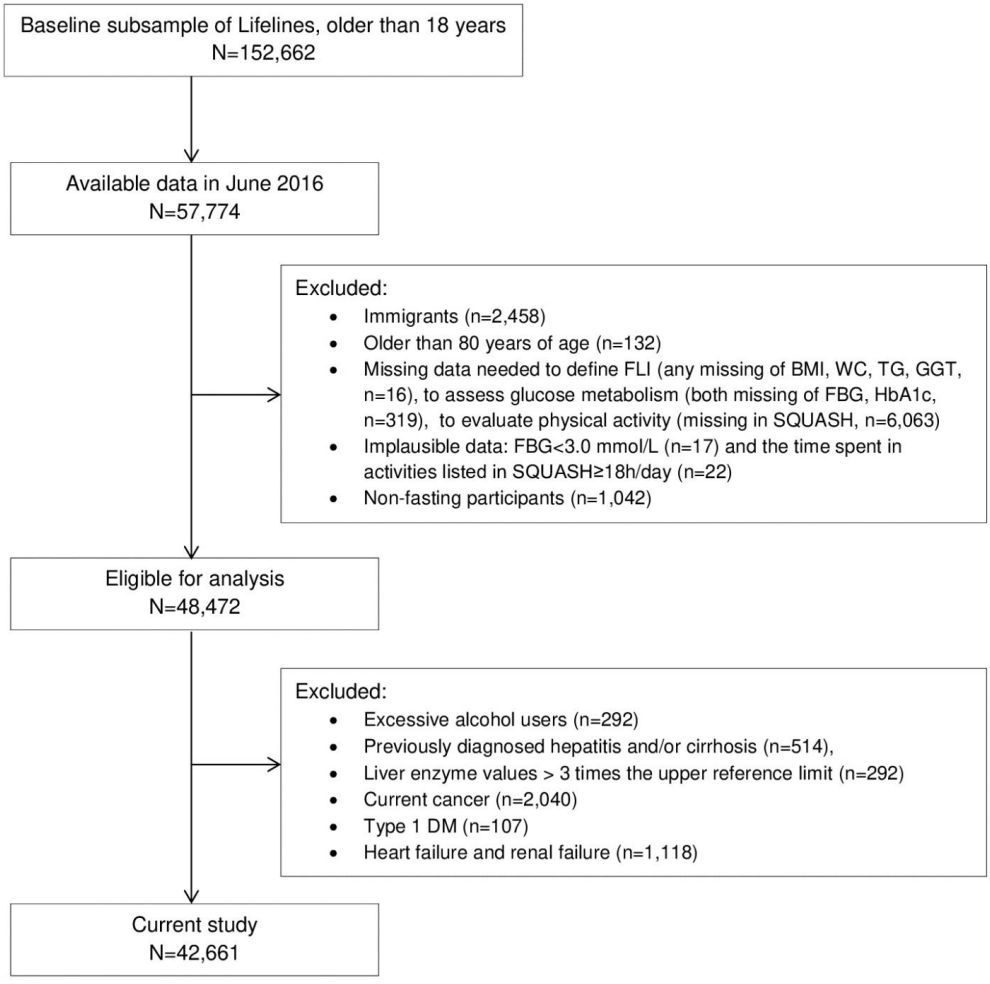

Figure S1. Flowchart of the study population

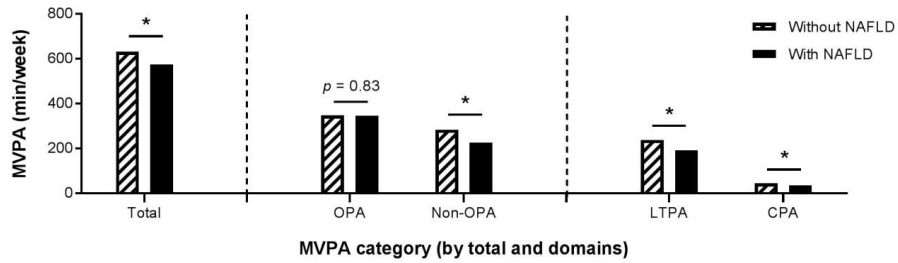

Figure S2. Daily-life moderate-to-vigorous physical activity, according to the presence of NAFLD.

Note: Data are presented as minutes per week adjusted for age, sex and education. Non-occupational MVPA included commuting and leisure-time physical activity.

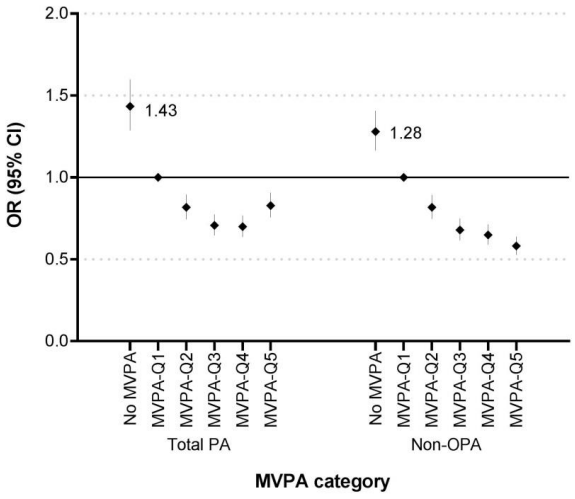

Figure S3. Sensitivity analysis for the association between MVPA categories and the risk of having NAFLD.

Note: Binary logistic regression analysis. Data are presented as odds ratio (95% CI). Error bars indicate 95% confidence interval (95%CI). References were each 'No MVPA' group from the six categories of total and non-occupational daily-life MVPA respectively in the analyses. Analysis was adjusted for age, sex and education, smoking and daily caloric intake. OR, odds ratio; MVPA, moderate to vigorous activity; Q=quintile.